

**This Page Is Inserted by IFW Operations
and is not a part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problems Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/00, 38/16, 39/395, C07K 14/00, 14/715, 14/435, 16/00, 16/24, 16/28		A1	(11) International Publication Number: WO 96/22785 (43) International Publication Date: 1 August 1996 (01.08.96)
(21) International Application Number: PCT/US96/01150		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 23 January 1996 (23.01.96)		Published <i>With international search report.</i>	
(30) Priority Data: 08/377,077 23 January 1995 (23.01.95) US 08/454,042 30 May 1995 (30.05.95) US			
(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).			
(72) Inventors: BROADDUS, Virginia, C.; 4020 23rd Street, San Francisco, CA 94114 (US). MATTHAY, Michael, A.; 1998 16th Avenue, San Francisco, CA 94116 (US). FOLKESSON, Hans, G.; 700 Parnassus Avenue, No. 14, San Francisco, CA 94122 (US).			
(74) Agent: BERLINER, Robert; Robbins, Berliner & Carson, 5th floor, 201 N. Figueroa Street, Los Angeles, CA 90012-2628 (US).			

(54) Title: METHODS FOR THE TREATMENT OF ACID ASPIRATION-INDUCED ACUTE LUNG INJURY

(57) Abstract

Lung injury from acid aspiration is treated by administering an IL-8-binding substance, such as anti-IL-8-antibody, to the patient following the acid aspiration.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**METHODS FOR THE TREATMENT OF ACID
ASPIRATION-INDUCED ACUTE LUNG INJURY**

5

This invention was made with Government support under Grant Nos. HL 19155 and HL 51854, awarded by the National Institutes of Health. The Government has certain rights in this invention.

10

The present invention is a continuation-in-part of Application Serial No. 08/377,077, filed on January 23, 1995, the full disclosure of which is incorporated herein by reference.

15

BACKGROUND OF THE INVENTION

1. Field of the Invention

20

The present invention relates to a method for treating and protecting patient lungs exposed to acid resulting from the aspiration of gastric contents or other causes.

25

The aspiration of gastric acid can occur, for example, when an unconscious patient regurgitates the stomach contents into the lungs. Acid aspiration is the second leading cause of the adult respiratory distress syndrome (ARDS), a syndrome which is characterized by injury to the pulmonary endothelium and the alveolar epithelium with leakage of blood and plasma into the interstitial and intra-alveolar spaces. The mortality from acid-induced ARDS range from 40% to 50%. No adequate treatments are presently available.

30

For these reasons, it would be desirable to provide methods for the treatment of patients exposed to acid aspiration into the lungs and at risk of developing ARDS. It would be further desirable if the methods could reduce damage to the pulmonary endothelium and alveolar epithelium, particularly when the treatment is given within one hour following the acid injury.

2. Description of the Background Art

Mulligan et al. (1993) J. IMMUNOL. 150:5585 describes the use of anti-IL-8 antibodies to treat immunologically induced lung inflammation. Sekido et al. 5 (1993) NATURE 365:654 shows that anti-IL-8 antibodies are able to reduce reperfusion injuries in ischemic lung tissue in rabbits. Anti-IL-8 antibodies have been shown to inhibit neutrophil influx in an endotoxin-induced pleurisy model in rabbits. Broaddus, et al. (1994) J. 10 IMMUNOL. 152:2960-2967. Elevated levels of IL-8 are present and cause neutrophil influx in patients suffering from adult respiratory distress syndrome. Miller et al. (1992) AM. REV. RESPIR. DIS. 146:427-432. The neutralization of TNF- α (which leads to IL-8 production in many cells) 15 has been shown to reduce lung injury caused by acid aspiration. Goldman et al. (1990) ANN. SURG. 212:513-520. A possible mechanism of acid aspiration-induced lung injury relies on the release of a variety of chemotactic and inflammatory molecules. The chemotactic and 20 inflammatory molecules, in turn, recruit neutrophils which induce injury upon binding to or migrating through the pulmonary capillary endothelium. Such a mechanism is discussed in a number of publications, including Ishii et al. (1989) PROSTAGLANDINS LEUKOT. ESSENT. FATTY ACIDS 37:65-70; 25 Goldman et al. (1990), *supra.*; Goldman et al. (1991) J. APPL. PHYSIOL. 70:1511-1517; Goldman et al. (1992) SURGERY 111:55-61; Fowler et al. (1987) AM. REV. RESPIR. DIS. 136: 1225-1231; Kindt et al. (1991) J. APPL. PHYSIOL. 70:1575- 1585; and Miller et al. (1992), *supra.* IL-8 has been 30 proposed as a major chemotactic factor for recruitment of neutrophils to extravascular sites of inflammation, including those in the lungs. Colditz et al. (1990) J. LEUKOC. BIOL. 48:129-137 and Kunkel et al. (1991) EXP. LUNG RES. 17:17-23.

SUMMARY OF THE INVENTION

According to the present invention, the magnitude of acute lung injury following acid aspiration is reduced or eliminated by the systemic administration 5 of an interleukin-8 (IL-8)-binding substance to the patient. The IL-8-binding substance is selected to neutralize free-IL-8 released from cells exposed to the acid. The neutralization of IL-8 inhibits or prevents 10 influx of neutrophils from circulation into the extravascular space of the lung, and it is believed that reduction of neutrophil recruitment by the IL-8-binding substance lessens the cellular damage. The exemplary IL- 15 8-binding substance is anti-IL-8 antibody. The IL-8-binding substance is administered to the patient as rapidly as possible following acid aspiration, but such administration can be delayed for as long as one hour without a substantial loss of therapeutical effectiveness.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and 1B. Alveolar-arterial oxygen tension difference in the positive control, pretreatment, treatment, and negative control groups over 6 h (Fig. 1A) and over 24 h (Fig. 1B). In the 6 h experiments, the 25 alveolar-arterial oxygen tension difference in the pretreatment and treatment groups was significantly less than that in the positive control group from 2 h onwards and was no different from that in the negative control group. In the 24 h experiments, the alveolar-arterial 30 oxygen tension difference was significantly less in the treatment (long-term) group than in the positive control (long-term) group by 2 h and remained low for 24 h. All the rabbits (n = 3) in the positive control (long-term) group died at 12-14 h. Data are means \pm SEM, * p<0.05 35 versus the negative control group (Fig. 1A) or the treatment (long-term) group (Fig. 1B), † p<0.05 versus

the pretreatment group (Fig. 1A), $^a p<0.05$ versus the treatment group (Fig. 1A).

Figs. 2A and 2B. The extravascular lung water (a quantitative index of pulmonary edema) in the positive 5 control, pretreatment, treatment, and negative control groups at 6 h (Fig. 2A) and in the positive control (long-term) group at 12-14 h and treatment (long-term) group at 24 h (Fig. 2B). In the short-term studies, the extravascular lung water in the pretreatment and 10 treatment groups was 35% less than in the positive control groups and no different from that in the negative control group. The extravascular lung water of a normal uninjected rabbit lung is 3.2 g water/g dry lung. In the long-term studies, the extravascular lung water was 15 100% lower in the treatment (long-term) group than in the positive control (long-term) group. Data are means \pm SD, $^a p<0.05$ versus the positive control group (Fig. 2A) or the positive control (long-term) group (Fig. 2B).

Figs. 3A and 3B. Endothelial permeability in 20 the lung measured as the accumulation of the vascular protein tracer, ^{131}I -albumin, in the extravascular spaces of the lung and expressed as extravascular plasma equivalents in the positive control, pretreatment, treatment, and negative control groups at 6 h (Fig. 3A), 25 and in the positive control (long-term) group at 12-14 h and the treatment (long-term) group at 24 h (Fig. 3B). In the short-term studies, the extravascular plasma equivalents were decreased by 70% in the pretreatment and treatment groups compared to the positive control group 30 at 6 h. The same reduction in extravascular plasma equivalents was observed in the treatment (long-term) group at 24 h compared to the positive control (long-term) group at 12-14 h. Data are means \pm SD, $^a p<0.05$ versus the positive control group (Fig. 3A) or the 35 positive control (long-term) group (Fig. 3B), $^b p<0.05$ versus the negative control group (Fig. 3A).

Figs. 4A and 4B. The number of neutrophils lavaged from the air spaces of rabbits in the positive control, pretreatment, treatment, and negative control groups at 6 h (Fig. 4A) and in the positive control 5 (long-term) group at 12-14 h and the treatment (long-term) group at 24 h (Fig. 4B). In the short-term studies, the number of neutrophils was 50% lower in the pretreatment and treatment groups than in the positive control group and no different from that in the negative 10 control group at 6 h. In the long-term studies, the number of neutrophils was more than 75% lower in the treatment (long-term) group at 24 h than in the positive control (long-term) group at 12-14 h. Data are means \pm SD, $p<0.05$ versus the positive control group (Fig. 4A) or 15 the positive control (long-term) group (Fig. 4B).

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention is based at least in part on the discovery that acid-induced lung injury is 20 mediated by neutrophils recruited to the lung by interleukin-8 (IL-8)-dependent mechanisms. It is believed that direct injury of the lung from the aspirated gastric acid is limited in extent, perhaps because the acid is rapidly neutralized after entering 25 the lungs. Acid aspiration, however, stimulates the release of substantial quantities of IL-8 into the airspaces from a variety of cells in the lung, including airway epithelial cells, alveolar epithelial cells, and alveolar macrophages. Once generated in the airspaces, 30 IL-8 is believed to diffuse toward the pulmonary endothelium, thereby establishing a chemotactic gradient for neutrophils and possibly binding to the luminal endothelial surface where the IL-8 could interact with circulating neutrophils. The interaction of IL-8 with 35 neutrophils then induces up regulation of neutrophil adhesion molecules on the endothelium, neutrophil migration through the activated endothelium, and priming

of neutrophils for activations by other mechanisms. Neutrophils recruited to the lung in this manner are believed to be responsible for most of the endothelial injury that results in formation of a protein-rich 5 pulmonary edema fluid that is characteristic of acid-aspiration acute lung injury.

The present invention relies on the binding of free IL-8 released from acid-stimulated airway epithelial cells, alveolar epithelial cells, and alveolar 10 macrophages. Binding preferably occurs through a region of the IL-8 molecule which binds to the IL-8-receptor on the neutrophils, thus directly blocking binding and recruitment of the neutrophils by the released IL-8. Binding should occur with an affinity of at least about 15 10^7 M^{-1} , preferably at least 10^8 M^{-1} .

Suitable binding substances for inhibiting binding between IL-8 and circulating neutrophils include anti-IL-8 antibodies, and fragments thereof; an IL-8-receptor protein, or fragment or analogue thereof; or any 20 other protein, glycoprotein, carbohydrate, small molecule, or the like, which is able to disrupt binding between the released IL-8 and the circulating neutrophils. The IL-8-binding substance will preferably bind to the IL-8 molecule at or adjacent to the region on 25 the molecule which binds to the IL-8-receptor on the neutrophils. By binding directly to this region, all interaction between the IL-8 molecule and the neutrophil will be blocked. By binding close to this region, sufficient stearic hinderance may be provided in order to 30 effectively inhibit neutrophil recruitment by the released IL-8.

The presently preferred IL-8-binding substance is neutralizing antibody to the IL-8 molecule. By "neutralizing," it is meant that the antibody will be 35 able to neutralize, i.e. inhibit, binding between the IL-8 molecule and the circulating neutrophils. Such antibodies may be prepared by employing IL-8, or an IL-8-

fragment, as an immunogen in conventional techniques for preparing polyclonal or monoclonal antibodies. Such techniques are well described in the scientific and patent literature. See, for example, *Antibodies: A 5 LABORATORY MANUAL*, Harlow and Lane Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). A particular technique for preparing neutralizing monoclonal antibodies is described in Broaddus, et al. (1994) *J. IMMUNOL.* 152:2960-2967.

10 The IL-8-binding substance will be systemically, usually intravenously, administered to the patient as quickly as possible after occurrence of the acid aspiration. Depending on the nature of the particular substance being administered, the total dosage 15 may vary from 1 μ g/kg of body weight to 10 mg/kg of body weight. In the exemplary case of monoclonal antibodies, the dosage will typically vary from 1 mg/kg to 10 mg/kg, with the total dosage being administered continuously, as a single bolus or as multiple boluses over time. While 20 intravenous administration is preferred, the IL-8 binding substance could be administered by other systemic routes, such as intratracheally.

Depending on the intended route of delivery, the IL-8-binding substance will be incorporated into a 25 suitable pharmaceutical composition including the desired dosage of the binding substance. Such compositions will usually include a pharmaceutically acceptable carrier, which can be any compatible, non-toxic substance suitable to deliver the IL-8-binding substance to the patient. 30 Sterile water, alcohol, fats, waxes, and inert solids, may be used as the carrier. Pharmaceutically acceptable adjuvants, buffering agents, and the like, may also be incorporated into the pharmaceutical compositions. Such compositions will be suitable for parenteral 35 administration, i.e. intravenous or for pulmonary (intratracheal) delivery. The preparation of such pharmaceutical compositions is well known in the art and

described in references, such as Remington's PHARMACEUTICAL SCIENCES, Gennaro, Ed., Mack Publishing Co., Easton, Pennsylvania 18042, 18th Ed., 1990.

5 The following examples are offered by way of illustration, not by illustration, not by way of limitation.

EXPERIMENTAL

METHODS

10 **Animals, Surgical Preparations and Ventilation**

Male New Zealand White rabbits (n = 32, weighing 2.5-3.5 kg, Nitabell, Hayward, CA., USA) were surgically prepared as described earlier. Folkesson et al. (1994) AM. J. RESPIR. CRIT. CARE MED. 150:1482-1486.

15 Briefly, the rabbits were initially anesthetized using 4% halothane in 100% O₂; the anesthesia was then maintained with 0.8% halothane in 100% O₂. Pancuronium bromide (0.3 mg/h x kg body weight; Pavulon®, Organon Inc., West Orange, N.J., USA) was given intravenously for

20 neuromuscular blockade.

A 22-gauge Angiocath® was inserted in the marginal ear vein for administering fluid and drugs. A PE-90 catheter was inserted in the right carotid artery to monitor systemic blood pressure and to obtain blood samples. A 4.0 mm ID endotracheal tube was inserted through a tracheostomy. The rabbits were maintained in the prone position during the experiments and ventilated with a constant-volume piston pump (Harvard Apparatus Co., Dover, MA., USA) with an inspired oxygen fraction of 1.0 and with a peak airway pressure of 15-18 cm H₂O during the baseline period, and supplemented with a positive end-expiratory pressure of 4 cm H₂O. During the baseline period, the respiratory rate was adjusted to maintain the arterial PCO₂ between 35-40 mm Hg. Thereafter, the ventilator settings were kept constant throughout the experiment.

Preparation of the Instillate

A solution of 100 mOsm/kg of NaCl (1/3 normal saline) was prepared with isotonic 0.9% saline and distilled water. The 1/3 normal osmolality was chosen to 5 match the osmolality of gastric aspirates. Then, hydrochloric acid (HCl) was added to the solution and titrated to a pH of 1.5. In the negative control studies, 1/3 normal saline was used as the instillate. 10 Evans blue dye (1 mg, Aldrich Chemical Company Inc., Milwaukee, WI., USA) was added to all instillates in order to confirm at post-mortem examination that the instilled fluid was distributed equally to both lungs.

Generation of the Monoclonal Antibody to Rabbit rIL-8

15 The generation of the monoclonal antibody to rabbit rIL-8 (ARIL8.2) has been described in detail. Broaddus et al. (1994) J. IMMUNOL. 152:2960-2967. ARIL8.2 was used because of its ability to recognize rabbit IL-8, to inhibit binding of ^{125}I -labeled rabbit rIL-8 to the IL-8 20 receptor, to block rabbit rIL-8-induced signal transduction via the IL-8 receptor, and to inhibit rabbit rIL-8-induced chemotactic activity for rabbit neutrophils. ARIL8.2 had a high affinity for rabbit IL-8 ($K_d = 0.42 \text{ nM}$). ARIL8.2 did cross-react with human IL-8, 25 but not with closely related cytokines (hMGSA, platelet factor-4, β -thromboglobulin), other human cytokines (IL-1 β , TNF- α), or other chemotactic factors (FMLP, C5a). The antibody preparation was sterile filtered and endotoxin was undetectable by Limulus assay.

30

General Experimental Protocol

In all experiments, after the surgical preparations, a 1 h baseline of stable heart rate, systemic blood pressure, and arterial blood gases was 35 required before the instillation. Fifteen minutes into the baseline period, 3 μCi ^{131}I -labeled human serum albumin (^{131}I -albumin, Frosst Laboratories) was injected

intravenously as a vascular tracer protein. Blood samples were obtained every 15 minutes for the remaining 45 minutes of the baseline period. The vascular tracer was used to calculate the flux of plasma protein into the 5 extravascular spaces of the lung.

For the instillation, a tubing (5 Fr., Accumark® Premarked Feeding Catheter, Concord/Portex, Keene, NH., USA) was gently passed through the tracheal tube until it was placed approximately 1 cm above the 10 carina. Then, HCl or 1/3 normal saline (4 ml/kg body weight) was instilled into both lungs over 3 minutes. After the instillation was completed, the tubing was withdrawn.

Thirty minutes after the instillation and 15 hourly thereafter during the 6 h or 24 h experimental period, blood was sampled.

At the end of the 6 h or 24 h experiments, the abdomen was opened and the rabbit was exsanguinated by transection of the abdominal aorta. The lungs were 20 removed through a median sternotomy. An alveolar sample was aspirated via sampling catheter gently passed through the trachea to a wedged position in a distal airway. Then, the left lung was clamped at the main bronchus for later use in extravascular lung water and tracer protein 25 measurements (see below). The right lung was then lavaged 2 times, using 6 ml of isoosmolar 0.9% NaCl containing 12 mM lidocaine (Sigma Biochemicals, St. Louis, MO., USA) each time.

The radioactivity of the samples was measured. 30 Total and differential cell counts were measured on the blood and bronchoalveolar lavage samples. The cells were counted as cells per ml lavage and then multiplied by the lavage column used (12 ml). Free, unbound IL-8 concentrations were measured in the plasma samples and 35 the alveolar samples.

By trichloracetic acid (TCA) precipitation of the instillates and selected samples from each

experiment, it was established that the vascular tracer ^{131}I remained more than 98% bound to protein.

Specific Experimental Protocol

5 There were six experimental groups. Three of these groups received HCl instillation and one received 1/3 normal saline instillation.

10 In the positive control group (n = 10), five minutes before the HCl instillation, the rabbits received either 0.9% NaCl (2 ml/kg body weight) or an irrelevant monoclonal antibody (2 mg/kg body weight) intravenously. The irrelevant monoclonal antibody was of the same isotype as ARIL8.2 (IgG2a) and directed against the gp120 envelope protein on the human immunodeficiency virus.

15 Because there were no differences in the studied parameters, the rabbits given the irrelevant monoclonal antibody and those given NaCl intravenously were combined into one group.

20 In the pretreatment group (n = 6), five minutes before the HCl instillation, the rabbits received the monoclonal antibody against IL-8 (ARIL8.2, 2 mg/kg body weight) intravenously.

25 In the treatment group (n = 6), one hour after the HCl instillation, the rabbits received ARIL8.2 (2 mg/kg body weight) intravenously.

30 In the negative control group (n = 4), five minutes before the 1/3 normal saline instillation, the rabbits received 0.9% NaCl (2 ml/kg body weight) intravenously.

35 In the positive control (long-term) group (n = 3), one hour after the HCl instillation, the rabbits received the irrelevant monoclonal antibody, anti-gp120 (2 mg/kg body weight) intravenously. The experiments were planned for 24 h, however all rabbits in this group died between 12-14 h after the HCl instillation.

In the treatment (long-term) group (n = 3), one hour after the HCl instillation, the rabbits received

ARIL8.2 (2 mg/kg body weight) intravenously. The rabbits were then studied for 24 h.

Hemodynamics, Airway Pressure, and Arterial Blood Gases

5 The heart rate, systemic blood pressure, and airway pressure were measured using calibrated pressure transducers (Pd23 ID, Gould Oxnard, CA., USA) and recorded continuously on a Grass polygraph (Grass Model 7 Polygraph, Grass Instruments, Quincy, MA., USA).
10 Arterial blood gases and pH and the systemic arterial pressure were measured every 30 minutes. The alveolar-arterial oxygen difference was calculated.

Extravascular Lung Water

15 Our method for the determination of extravascular lung water has been described previously in detail. Berthiaume et al. (1987) J. CLIN. INVEST. 79:335-343, and Wiener-Kronish et al. (1991) J. CLIN. INVEST. 88:864-875. In brief, the left lung was homogenized and
20 the extravascular lung water was determined by measuring the extravascular water-to-dry weight ratio (gram water/gram dry lung). Because the right lung was lavaged for cell counts, the data for extravascular lung water was obtained for the left lung only. The bronchoalveolar
25 lavage from the right lung and the homogenates from both lungs were used for measurement of radioactivity (see below).

Lung Vascular Permeability

30 For measurement of lung endothelial permeability to protein, the clearance of the vascular tracer protein, ¹³¹I-albumin, across the endothelium into the extravascular compartments of the lungs was measured. The total extravascular ¹³¹I-albumin accumulation in the
35 lung was calculated by taking the total lung ¹³¹I-albumin (in lung homogenate and in the alveolar samples) and subtracting the vascular space ¹³¹I-albumin. The ¹³¹I-

albumin in the vascular space was calculated by multiplying the counts in the final plasma sample by the calculated plasma volume in the lungs, as we have done previously. Berthiaume et al. (1987) J. CLIN. INVEST. 79:335-343, and Wiener-Kronish et al. (1991) J. CLIN. INVEST. 88:864-875. The extravascular accumulation of ¹³¹I-albumin in the lung was expressed as plasma equivalents, or the milliliters of plasma that would account for the radioactivity in the lung.

10

Measurement of Free IL-8 Concentrations

The concentrations of free IL-8, not bound by the anti-IL-8 monoclonal antibody, were measured by ELISA in plasma and in the final alveolar sample, as described. 15 Broaddus et al. (1994) J. IMMUNOL. 152:2960-2967. In this assay ARIL8.2 was used as the primary capture mAb so that it would not capture IL-8 already bound to ARIL8.2. Microtiter plates (96-well; ImmunoPlate MaxiSorp. Nunc. Alameda Chemical Sciences, Oakland, CA., USA) were coated 20 with ARIL8.2 (10 µg/ml), and then blotted dry and blocked with phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (Sigma) for 1 h. Standards of rrIL-8 mixed with ARIL8.2, plasma samples and alveolar samples in several dilutions were added to the wells for a 1 h 25 incubation. After washing, the secondary antibody (8Cl.1.6), conjugated to long-arm biotin (Biotin-S-NHS, Research Organics, Cleveland, OH., USA), was added for 2 h followed by horseradish-peroxidase-conjugated streptavidin (1:5,000; Zymed Laboratories, South San 30 Francisco, CA., USA) for 1 h. Tetramethyl benzidine (TMB; 2 component system, Kirkegaard & Perry Laboratories, Gaithersburg. MD., USA) was then added and color was allowed to develop in room temperature for 10 minutes. Optical density was then measured with an ELISA 35 plate reader at a wave length of 405 nm. Sample values were determined by interpolation using a 4-parameter program (Genentech Inc., South San Francisco, CA., USA)

from a standard curve generated over a range of 2,000 to 31 pg/ml. For the non-neutralized samples, the results for the dilutions were averaged over their linear range. However, when detecting antigen in the presence of a soluble antibody identical to the capture antibody, an ELISA can be nonlinear at increasing dilutions, perhaps because antigen dissociates from the solutable antibody and is subsequently bound by the capture antibody. Therefore, for the neutralized samples, we chose the lowest dilution (1:10) for quantitating free IL-8, knowing that this may still be an overestimate of the free IL-8 present. When testing the ELISA with standards of rrIL-8 mixed with ARIL8.2, we found that, as ARIL8.2 concentrations increased, the free IL-8 detected decreased until, at a molar ratio of 5:1 and higher (mAb:IL-8), no IL-8 could be detected. All samples were coded so that the experimental condition was not known by the individual doing the assays.

20 Statistical Analysis

One-way ANOVA with repeated measurements analysis was used to compare samples obtained at several time points from the same animal. One-way ANOVA (factorial) was used when comparing other single groups. Student-Newman-Keuls test was used as a post-hoc statistical test. Values are expressed as either mean \pm SD or mean \pm SEM as indicated in tables and figure legends. The data from the HCl instilled rabbits pretreated with either the irrelevant monoclonal antibody, anti-gp 120, or with saline were combined because there were no significant differences between the groups.

RESULTS

35 Oxygenation, Ventilation and pH

In the short-term experiments, the alveolar-arterial oxygen tension differences in the

ARIL8.2 pretreatment and treatment groups were significantly lower than in the positive control group by 2 h after the acid instillation and remained lower for the 6 h experiment (Fig. 1A and Table 1). In both the 5 ARIL8.2 pretreatment and treatment groups, the alveolar-arterial oxygen tension difference was not significantly different from that in the negative control group (Fig. 1A and Table 1).

Table 1. Oxygenation, ventilation, arterial pH, systemic blood pressure, heart rate, and airway pressure in the short-term experiments.

Condition	Alveolar-Arterial Oxygen Difference (mm Hg)	Arterial PCO ₂ (mm Hg)	pH	Mean Systemic Arterial Pressure (mm Hg)	Heart Rate (beats/min)	Peak Airway Pressure (cm H ₂ O)
Positive Control Group (HCl), n = 10						
Baseline	107 ± 31	37 ± 4	7.44 ± 0.06	68 ± 13	300 ± 30	17 ± 3
6 h after instillation	390 ± 137*	67 ± 18*	7.19 ± 0.13*	56 ± 21	285 ± 23	26 ± 8*
Pretreatment Group (ARIL8.2, HCl), n = 6						
Baseline	107 ± 35	36 ± 4	7.44 ± 0.05	61 ± 13	307 ± 38	17 ± 2
6 h after instillation	136 ± 48*	38 ± 8†	7.37 ± 0.04†	52 ± 10	276 ± 35	22 ± 4*
Treatment Group (HCl, ARIL8.2), n = 6						
Baseline	108 ± 40	32 ± 3	7.46 ± 0.08	63 ± 12	304 ± 34	16 ± 1
6 h after instillation	149 ± 40*	39 ± 5†	7.36 ± 0.03†	52 ± 18	288 ± 28	23 ± 3*
Neutrophil Depletion Group (HCl), n=4						
Baseline	110 ± 54	30 ± 3	7.47 ± 0.07	64 ± 15	301 ± 23	16 ± 2
6 h after instillation	241 ± 64*	33 ± 6†	7.36 ± 0.05†	53 ± 13	280 ± 19	22 ± 2*
Negative Control Group (1/3 Normal Saline), n = 4						
Baseline	109 ± 22	36 ± 2	7.41 ± 0.09	61 ± 4	300 ± 29	17 ± 2
6 h after instillation	108 ± 51†	41 ± 3 †	7.38 ± 0.05†	62 ± 12	287 ± 21	20 ± 2†

Data are means \pm SD
p<0.05 versus baseline
† p<0.05 versus positive control group

In the long-term experiments, the alveolar-arterial oxygen tension difference was significantly lower in the treatment (long-term) group than in the positive control (long-term) group by 2 h after HCl instillation (Fig. 1B and Table 2).
5 In the positive control (long-term) group, the rabbits died with severe hypoxemia between 12-14 h. In the treatment (long-term) group, on the other hand, the rabbits lived for 24 h without hypoxemia.

Table 2. Oxygenation, ventilation, arterial pH, systemic blood pressure, heart rate, and airway pressure in the long-term experiments.

Condition	Alveolar-Arterial Oxygen Difference	Arterial PCO ₂	pH	Mean Systemic Arterial Pressure (mm Hg)	Heart Rate (beats/min)	Peak Airway Pressure (cm H ₂ O)
Positive Control (long-term) Group (HCl), n = 3						
Baseline	110 ± 12	31 ± 2	7.42 ± 0.05	55 ± 16	292 ± 7	18 ± 2
6 h after instillation	484 ± 88*	68 ± 1*	7.22 ± 0.03*	59 ± 23	284 ± 18	28 ± 4*
12 h after instillation [†]	504 ± 115*	87 ± 19*	7.12 ± 0.10*	48 ± 7	256 ± 30	31 ± 5*
Treatment (long-term) Group (HCl, ARIL8.2), n = 3						
Baseline	112 ± 18	30 ± 4	7.42 ± 0.02	56 ± 8	284 ± 7	17 ± 1
6 h after instillation	152 ± 19 [†]	33 ± 2 [†]	7.36 ± 0.06 [†]	57 ± 4	288 ± 21	21 ± 1 [†]
12 h after instillation	122 ± 14 [†]	33 ± 2 [†]	7.34 ± 0.04 ^{††}	54 ± 4	280 ± 18 [†]	21 ± 1 [†]
24 h after instillation	161 ± 29 [†]	30 ± 2	7.36 ± 0.01 [†]	49 ± 6	272 ± 14	21 ± 3 [†]

Data are means ± SD

* p<0.05 versus baseline

† p<0.05 versus positive control (long-term) group
all rabbits in this group died between 12-14 h

The acid-induced abnormalities in PaCO₂ and pH were prevented by either pretreatment or treatment with ARIL8.2 both in the 6 h and 24 h studies as well as by neutrophil depletion (Table 1 and Table 2).

5

Extravascular Lung Water

In the short-term experiments, the extravascular lung water (water-to-dry weight ratio) in the pretreatment and treatment groups was 35% lower than 10 in the positive control group and not significantly different from that in the negative control group at 6 h (Fig. 2A). In the long-term experiments, the extravascular lung water in the treatment (long-term) group at 24 h was 100% lower than in the positive control 15 (long-term) group at 12-14 h (Fig. 2B).

Lung Vascular Permeability

In the short-term experiments, the extravascular accumulation of plasma equivalents in the 20 lungs of the pretreatment and treatment groups was 70% lower than in the positive control group at 6 h (Fig. 3A), although the extravascular accumulation of plasma was significantly higher than in the negative control group. In the long-term experiments, the extravascular 25 accumulation of plasma equivalents in the lung was 75% lower in the treatment (long-term) group at 24 h than in the positive control (long-term) group at 12-14 h (Fig. 3B).

30 Systemic Blood Pressure, Heart Rate, and Peak Airway Pressure

No differences were observed in the blood pressure or heart rate at any time among the experimental 35 groups (Table 1). The peak airway pressure rose in all groups within 5 minutes after instillation. While the airway pressure in the positive control group remained high, the airway pressure in the negative control group

decreased by 6 h. In both the pretreatment and treatment groups, the airway pressure tended to decrease, although this did not reach statistical significance (Table 1). Similar findings were observed in the long-term studies 5 (Table 2).

Cell Counts in Bronchoalveolar Lavage Fluid and in Peripheral Blood

In the short-term experiments, the number of 10 polymorphonuclear leukocytes (PMN) lavaged from the air spaces in the pretreatment and treatment groups was more than 50% lower than in the positive control group and no different from that in the negative control group (Fig. 4A). In the long-term experiments, the number of lavaged 15 neutrophils in the treatment (long-term) group at 24 h was more than 75% lower than in the positive control (long-term) group at 12-14 h (Fig. 4B). No significant differences were seen in the number of alveolar macrophages between the different groups (data not 20 shown). In the peripheral blood, there was an identical small increase in the neutrophil count in all groups, except in the neutrophil-depleted group, where no circulating neutrophils were observed at any time during the experiment.

25

Concentrations of Free Interleukin-8 Plasma, Alveolar Fluid, and Bronchoalveolar Lavage Fluid

In the short-term experiments, the concentrations of free IL-8 in the final alveolar fluid 30 samples were more than 10-fold lower in the pretreatment and treatment groups than in the positive control group at 6 h (Table 3). Because no undiluted alveolar fluid could be aspirated in the negative control group, free IL-8 was also measured in bronchoalveolar lavage fluid. 35 As was the case in the alveolar fluid, free IL-8 concentrations in the lavage fluid were more than 10-fold lower in the pretreatment and treatment groups than in

the positive control group (Table 3). Of interest, the free IL-8 in lavage fluid in the treatment group was not different from the negative control group (Table 3). In the long-term experiments, alveolar fluid could not be
5 aspirated from the treatment (long-term) group. However, in the lavage fluid, the free IL-8 concentrations were significantly lower in the treatment (long-term) group than in the positive control (long-term) group (Table 3). In the plasma, the concentration of immunoreactive free
10 IL-8 (not bound by the anti-IL-8 monoclonal antibody, ARIL8.2) was low at all times and similar for all groups (330 ± 28 pg/ml).

Table 3. Free rabbit IL-8 concentrations (IL-8 not bound by the anti-IL-8 monoclonal antibody, ARIL8.2) in alveolar fluid and lavage fluid in the various experimental groups.

Condition	Time of sampling	IL-8 concentrations in Alveolar Fluid (ng/ml)	IL-8 concentrations in Lavage Fluid (ng/ml)
Positive control group (HCl)	6 h	40.5 ± 18.2 (8)	6.7 ± 2.5 (4)
Pretreatment group, (HCl) (ARIL8.2)	6 h	3.3 ± 1.9* (6)	nc
Treatment group (HCl) (ARIL8.2)	6 h	3.0 ± 1.7* (6)	0.6 ± 0.3† (3)
Negative control group (1/3 normal saline)	6 h	na	1.4 ± 1.6* (3)
Positive control (long-term group (HCl)	12-14 h	29 ± 2.2 (3)	2.1 ± 2.8 (3)
Treatment (long-term) group (HCl, ARIL8.2)	24 h	na	0.2 ± 0.2† (3)

* p<0.05 versus positive control group;

† p<0.05 versus positive control (long-term) group

na sample not possible to obtain

nd not determined

Number of determinations within parenthesis

Values are means ± SD

DISCUSSION

The results of this study confirm that acid aspiration-induced lung injury is primarily mediated by 5 neutrophils recruited to the lung by IL-8. First, in the rabbits instilled with acid, large numbers of neutrophils were recovered from the air spaces in association with markedly elevated quantities of IL-8. The nearly undetectable concentrations of IL-8 in the plasma in all 10 groups supports the premise that IL-8 is generated

locally in the lung following acid aspiration. The concentrations of IL-8 in the air spaces 6 h after acid aspiration (41 ± 18 ng/ml.) are biologically relevant; in *in vitro* assays of chemotaxis and neutrophil priming, IL-8 is biologically active at ten-fold lower concentration. Second, the role of the neutrophil in injuring the lung was supported by the marked reduction of lung injury after neutrophil depletion. Third, the role of IL-8 in mediating the recruitment of neutrophils and the resultant lung injury following acid instillation was established by neutralizing IL-8. The anti-IL-8 antibody effectively reduced the concentrations of free IL-8 to less than 10% of the concentration in the positive control (acid-instilled) rabbits, to a level at the lower limit of the biological activity of IL-8 in both *in vitro* and *in vivo* studies. This IL-8 concentration was probably close to that in the negative control (saline-instilled) rabbits, as judged by the similar IL-8 concentrations in the bronchoalveolar lavage fluid. The use of the anti-IL-8 monoclonal antibody led to a more than 50% decrease in the neutrophil influx and, more importantly, to a dramatic reduction in the severity of the acute lung injury due to acid aspiration. Following neutralization of IL-8, the acid-induced abnormalities in gas exchange, extravascular lung water, and lung vascular permeability were nearly completely prevented.

The three separate indices of lung injury demonstrated internally consistent and convincing results in both the short-term (6 h) and the long-term (24 h) studies. First, the alveolar-arterial oxygen tension difference was nearly normal in acid-instilled rabbits given the anti-IL-8 monoclonal antibody, indicating the absence of alveolar edema. Second, when IL-8 was neutralized, the extravascular lung water in acid-instilled rabbits was not different from that in rabbits instilled with saline alone. The water-to-dry weight ratio of 4.2-4.4 g water/g dry lung in the pretreatment

and treatment groups and in the saline-instilled control group is most consistent with mild interstitial edema. In the positive control group, on the other hand, the water-to-dry weight ratio of 7.0 g water/g dry lung at 5 6 h and 8.0 g water/g dry lung at 12-14 h clearly indicates significant alveolar edema. These differences become more obvious when the lung water is expressed as the calculated milliliters of water accumulated in the lung in excess of that in a normal rabbit lung (3.2 g 10 water/g dry lung). In the positive control group at 6 h, the excess water in both lungs was approximately 9.2 ml, or more than three-fold higher than the amount (1.6-2.6 ml) in the negative control, pretreatment and treatment groups. Finally, the endothelial barrier was 15 significantly protected in the acid-instilled rabbits given the anti-IL-8 monoclonal antibody. There was a small increase in lung endothelial permeability that was not prevented by pretreatment or treatment with the monoclonal antibody to IL-8. This increase, however, was 20 not sufficient to cause a net accumulation in extravascular lung water. A small increase in lung endothelial permeability without an accompanying increase in lung water has previously been described in sheep given an endotoxin infusion. Overall, once IL-8 was 25 neutralized, the effects of acid on the lung were not different from the effects of saline alone.

The fact that the anti-IL-8 monoclonal antibody was equally effective when given 1 h after the acid instillation as when administered 5 minutes before the 30 injury confirms that the development of lung injury is delayed following acid instillation. Such a time course is consistent with the kinetics expected for IL-8 expression. Studies in which IL-8 concentrations have been measured sequentially *in vivo* demonstrate that 35 biologically relevant IL-8 concentrations are first found 2 h after an endotoxin stimulus. With additional time, IL-8 concentrations may be amplified further by the

action of proximal macrophage-derived cytokines such as TNF- α or IL-1 on the nearby bystander cells, such as epithelial cells. The important role of proximal cytokines in acid-induced lung injury has been suggested 5 by an earlier study in which neutralization of TNF- α significantly reduced the injury caused by acid-aspiration. Since TNF- α is a major proximal cytokine leading to the production of IL-8 by many cells, neutralization of TNF- α appears earlier than IL-8 in the 10 inflammatory cascade, it is unlikely that anti-TNF- α would be effective as late as anti-IL-8 following acid-instillation. Another factor that may contribute to the relatively wide therapeutic window of anti-IL-8 (at least 1 h) is that after its production, IL-8 must also diffuse 15 to the endothelium and interact with neutrophils. Given the time necessary for IL-8 production and diffusion, it is conceivable that anti-IL-8 therapy given later than 1 h after acid aspiration would also be effective.

Although the data in this study indicates that 20 IL-8 is critical for the development of the acute injury following acid aspiration, it is likely that IL-8 mediates injury in conjunction with other proinflammatory molecules. When used alone, IL-8 appears to be a relatively weak neutrophil activator, although *in vitro* 25 studies indicate that it is an effective primer of activation by other mechanisms. In *in vivo* studies in which IL-8 alone was injected into normal human skin, neutrophils were recruited without the appearance of wheal or flare. Similarly, when IL-8 alone was instilled 30 into a normal tracheal segment in dogs, neutrophils were recruited without releasing elastase or lysozyme. In contrast to these studies in which IL-8 was used alone, in acid aspiration lung injury, IL-8 is undoubtedly generated in conjunction with other cytokines, such as 35 TNF- α and IL-1, and other inflammatory mediators, such as leukotrienes, complement fragments, and platelet activating factor. However, in spite of the different

cytokines and inflammatory mediators generated following acid aspiration into the lung, inhibition of IL-8 alone is capable of preventing the experimental lung injury generated by acid aspiration in rabbits.

5 Aspiration of gastric contents is a major clinical cause of morbidity and mortality and effective therapy for this condition is currently unavailable. Current management is limited to positive pressure ventilation and careful management of fluid therapy. The
10 data in this study suggest a promising therapeutic potential for anti-IL-8 therapy for this condition. There are several possible advantages of this approach. Because IL-8 is a distal cytokine, its neutralization may have more limited effects than neutralization of a more
15 proximal, pluripotent cytokine, such as TNF- α . Also, neutralization therapy might be required only for a short time, during the time that IL-8 is generated. In in vivo studies following endotoxin stimulation, IL-8 concentrations have returned towards normal in less than
20 12 hours. In the long-term studies at 12-14 h following acid aspiration, alveolar fluid IL-8 concentrations in untreated rabbits were significantly lower than at 6 h, suggesting that a need for long-term anti-IL-8 therapy following a single acid aspiration might be unnecessary.
25 The time of onset of the aspiration would be known with certainty in many cases because gastric aspiration is frequently witnessed, whereas in other clinical conditions, such as sepsis, the time of onset is often difficult to identify. And, most importantly, the delay
30 in onset of the acid-aspiration injury allows a clinically feasible therapeutic window of at least one hour. A potential limitation of anti-IL-8 therapy is that, like any anti-inflammatory therapy, it might inhibit the host immunity and increase the risk of
35 infection. Indeed, secondary bacterial pneumonia is a known complication of acid aspiration, occurring 2-10 days after aspiration.

While the above is a complete description of the preferred embodiments of the invention, various alternatives, modifications, and equivalents may be used. Therefore, the above description should not be taken as limiting the scope of the invention which is defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method for treating a host having lungs exposed to an acid medium, said method comprising
5 administering to said host an amount of an anti-IL-8 binding substance effective to inhibit damage to the lungs resulting from such exposure.

10 2. A method as in claim 1, wherein the anti-IL-8 binding substance binds to the IL-8-receptor-binding region on IL-8.

15 3. A method as in claim 1, wherein the anti-IL-8 binding substance is anti-IL-8 antibody which binds to the IL-8-receptor-binding regions on IL-8.

4. A method as in claim 1, wherein the anti-IL-8 binding substance is administered intravascularly or by pulmonary delivery.

20 5. A method as in claim 1, wherein the anti-IL-8 binding substance is administered within one hour of exposure to acid medium.

25 6. A method as in claim 1, wherein the amount of anti-IL-8 binding substance is sufficient to neutralize IL-8 binding to neutrophils.

1/8

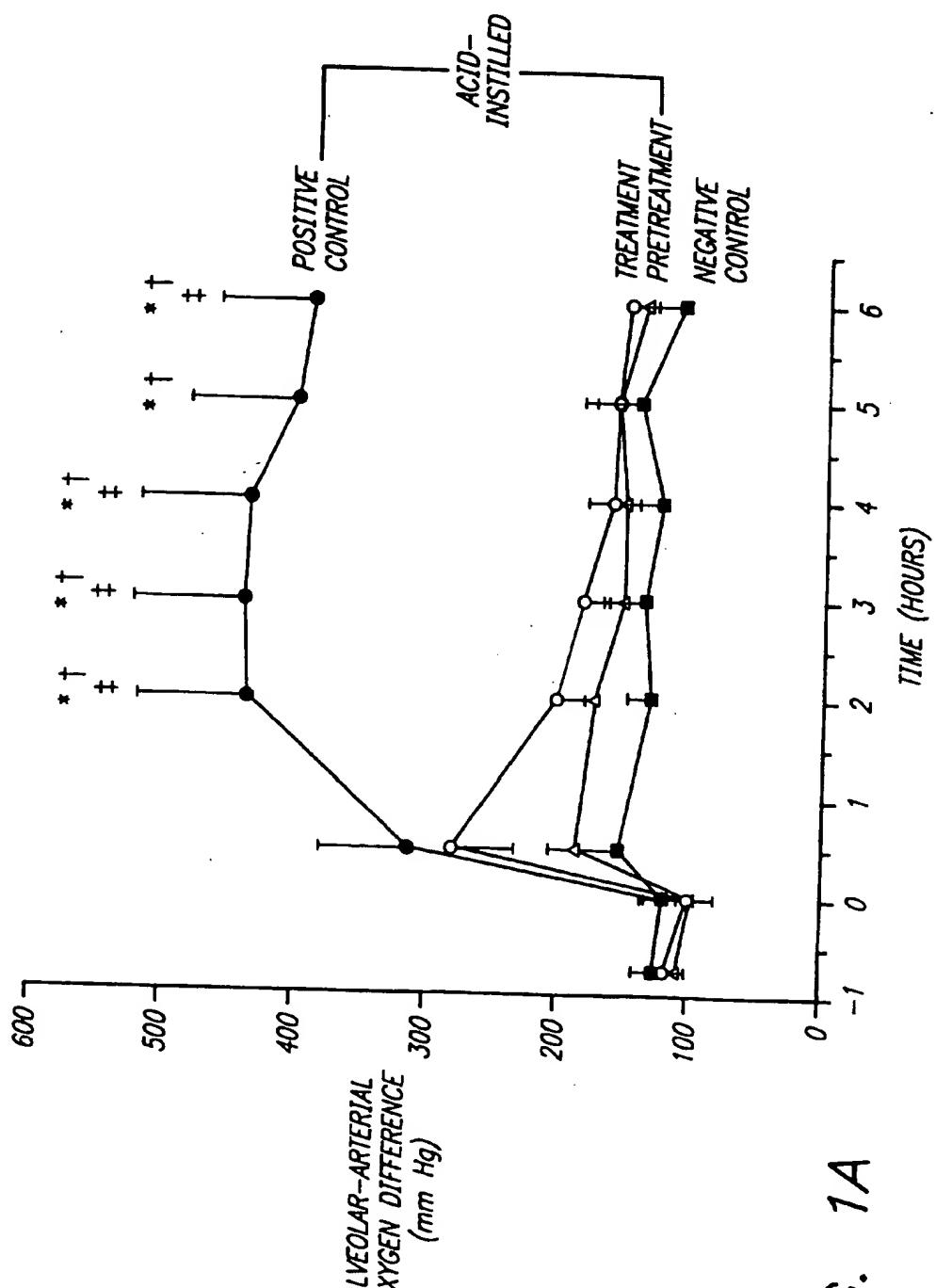


FIG. 1A

2/8

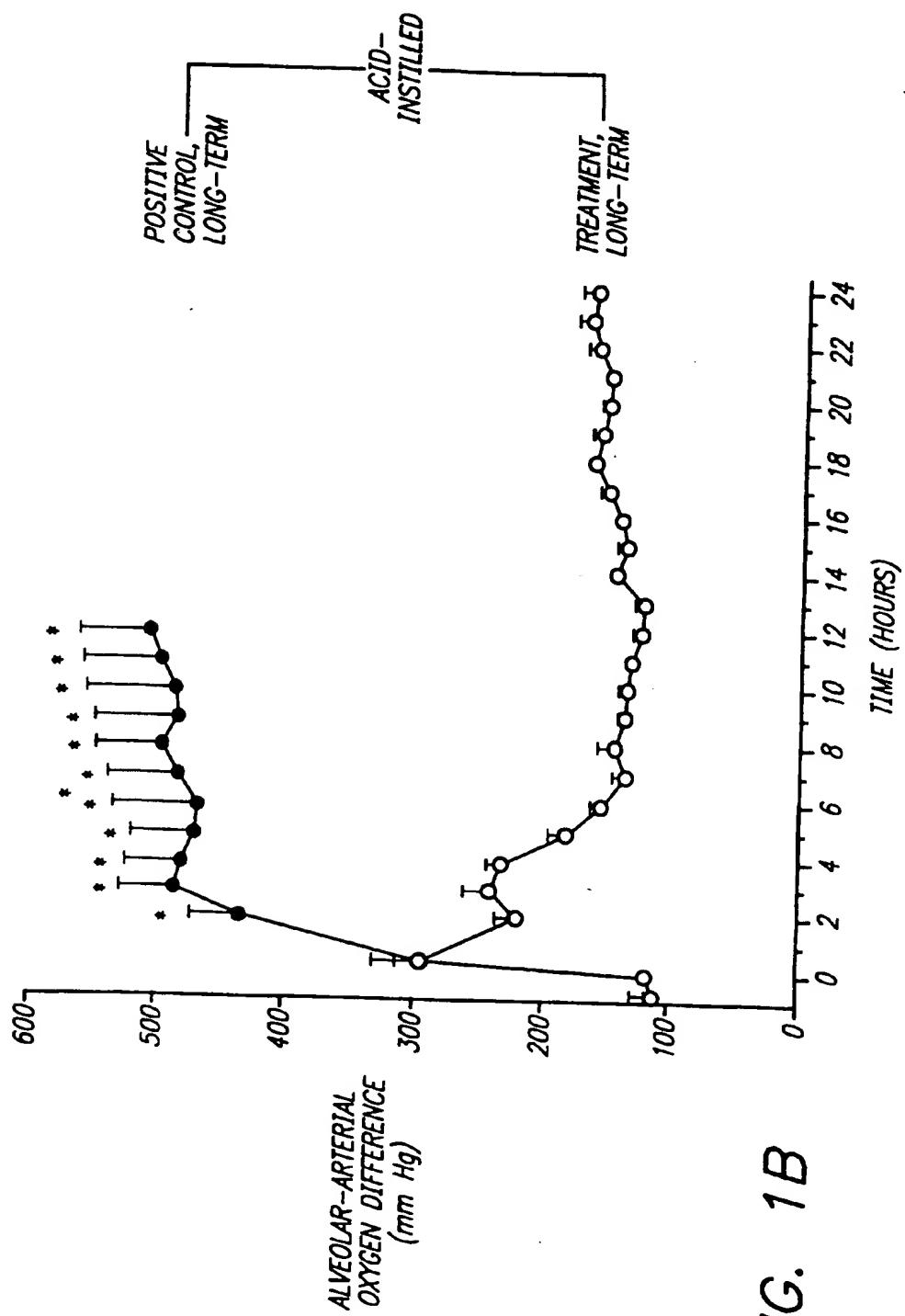
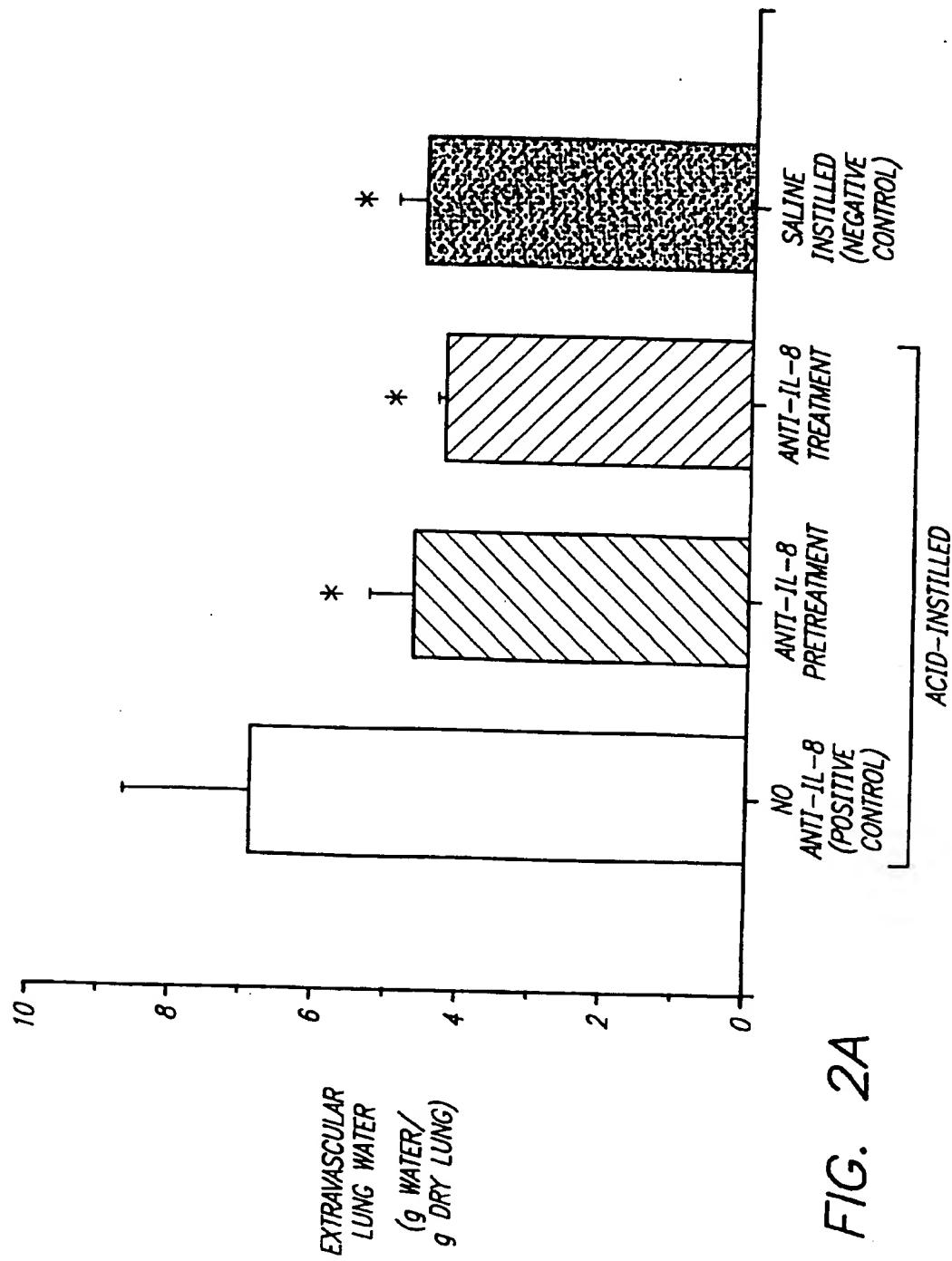


FIG. 1B

3/8



4/8

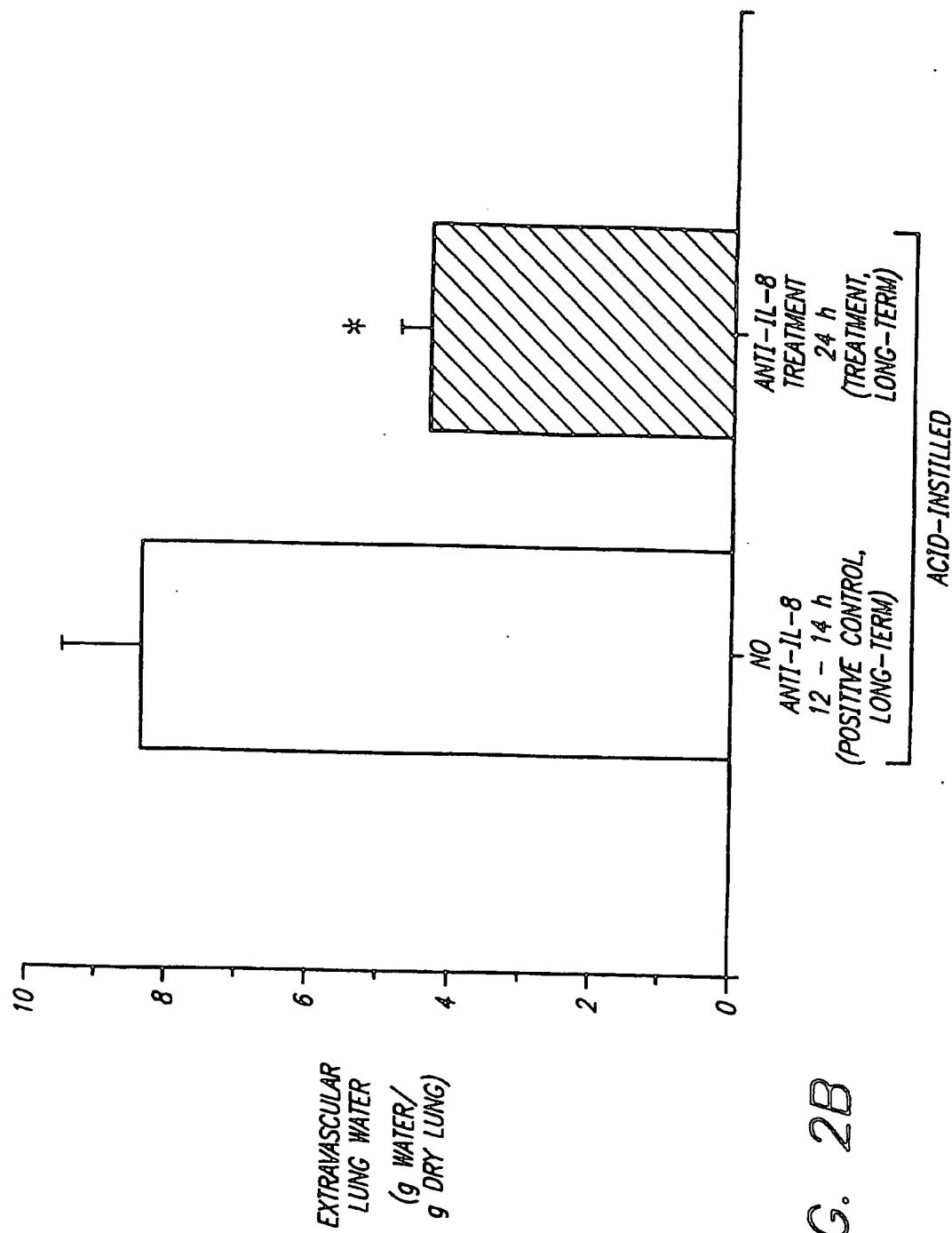


FIG. 2B

SUBSTITUTE SHEET (RULE 26)

5/8

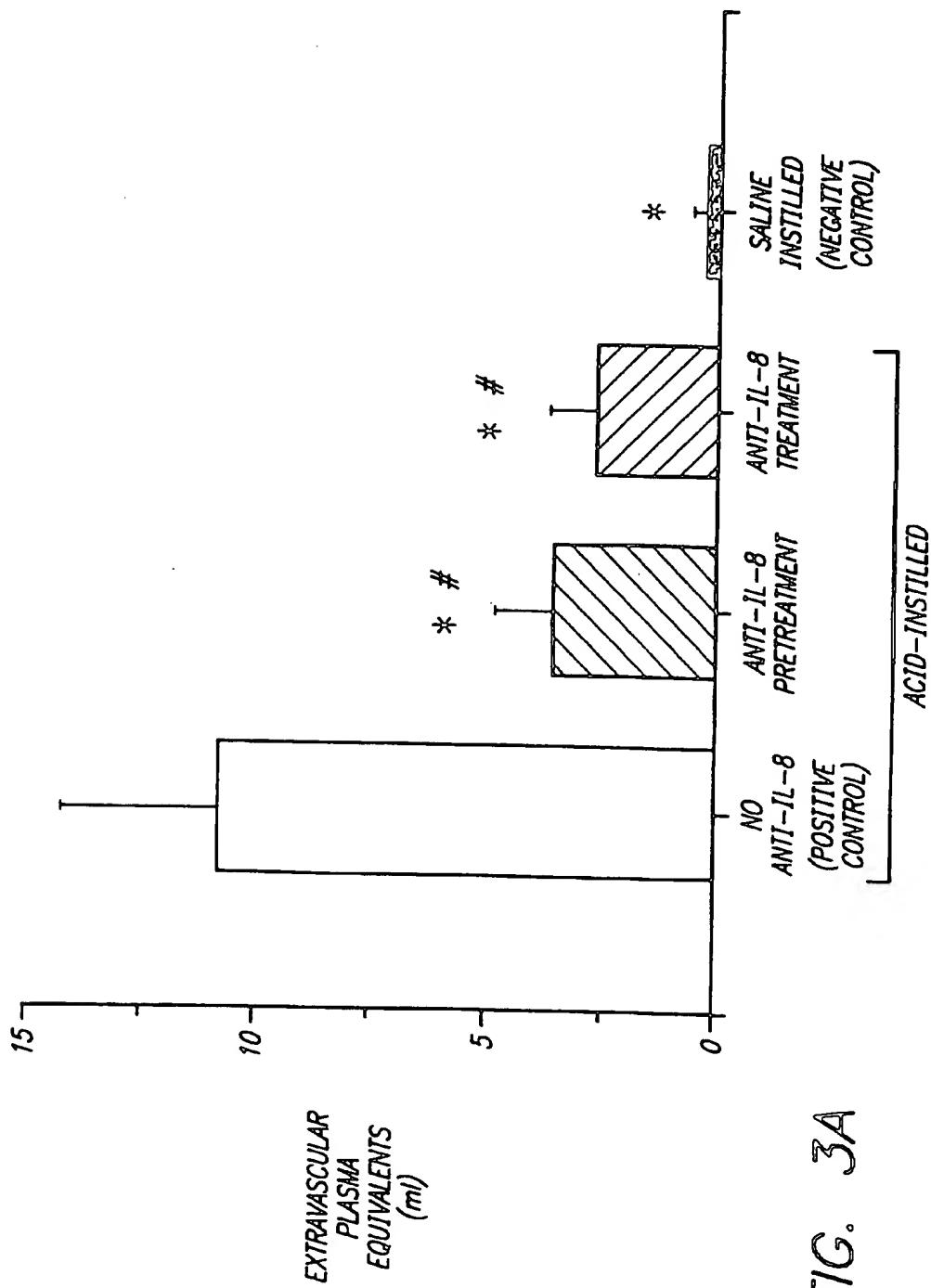


FIG. 3A

6/8

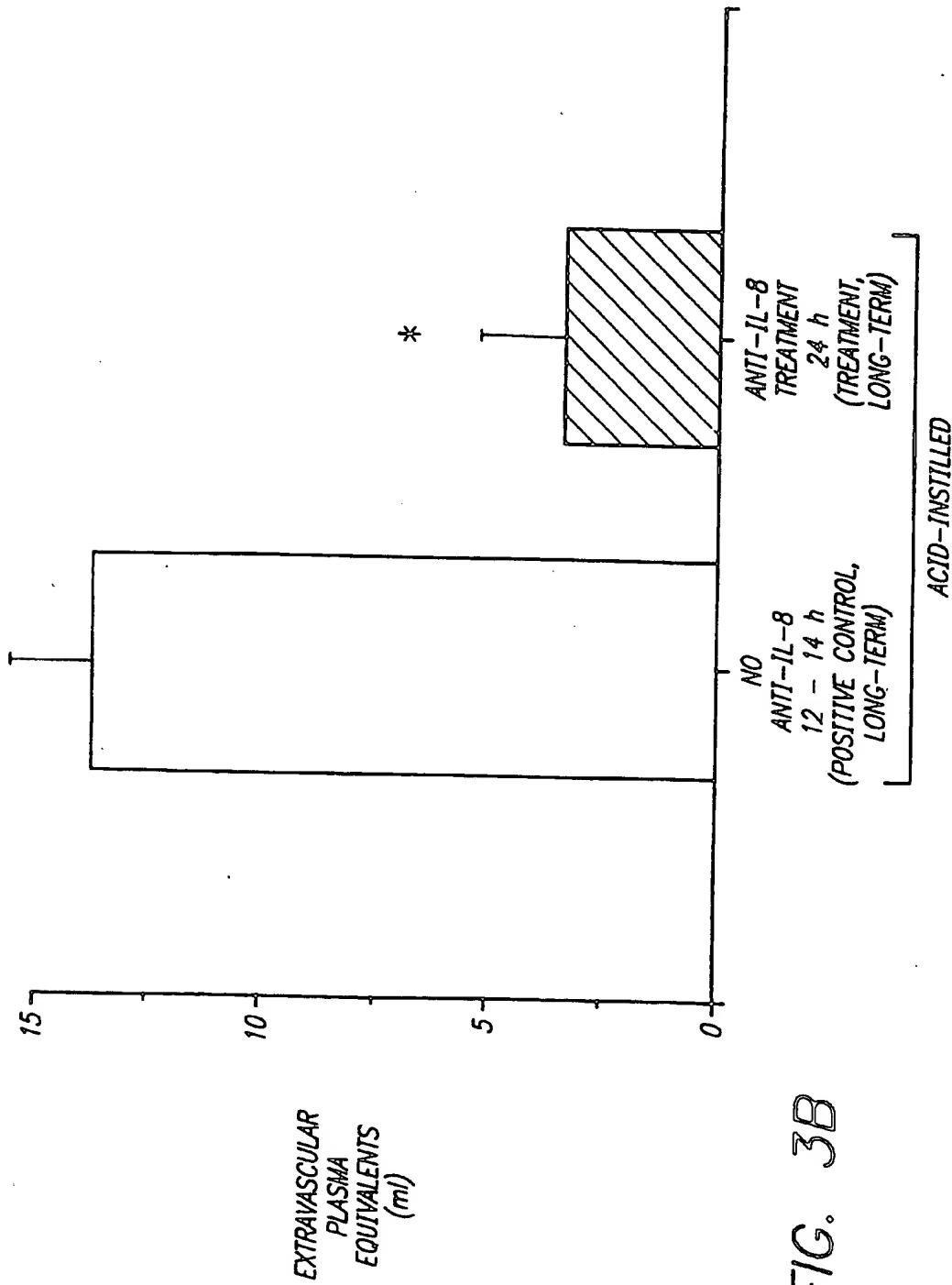


FIG. 3B

SUBSTITUTE SHEET (RULE 26)

7/8

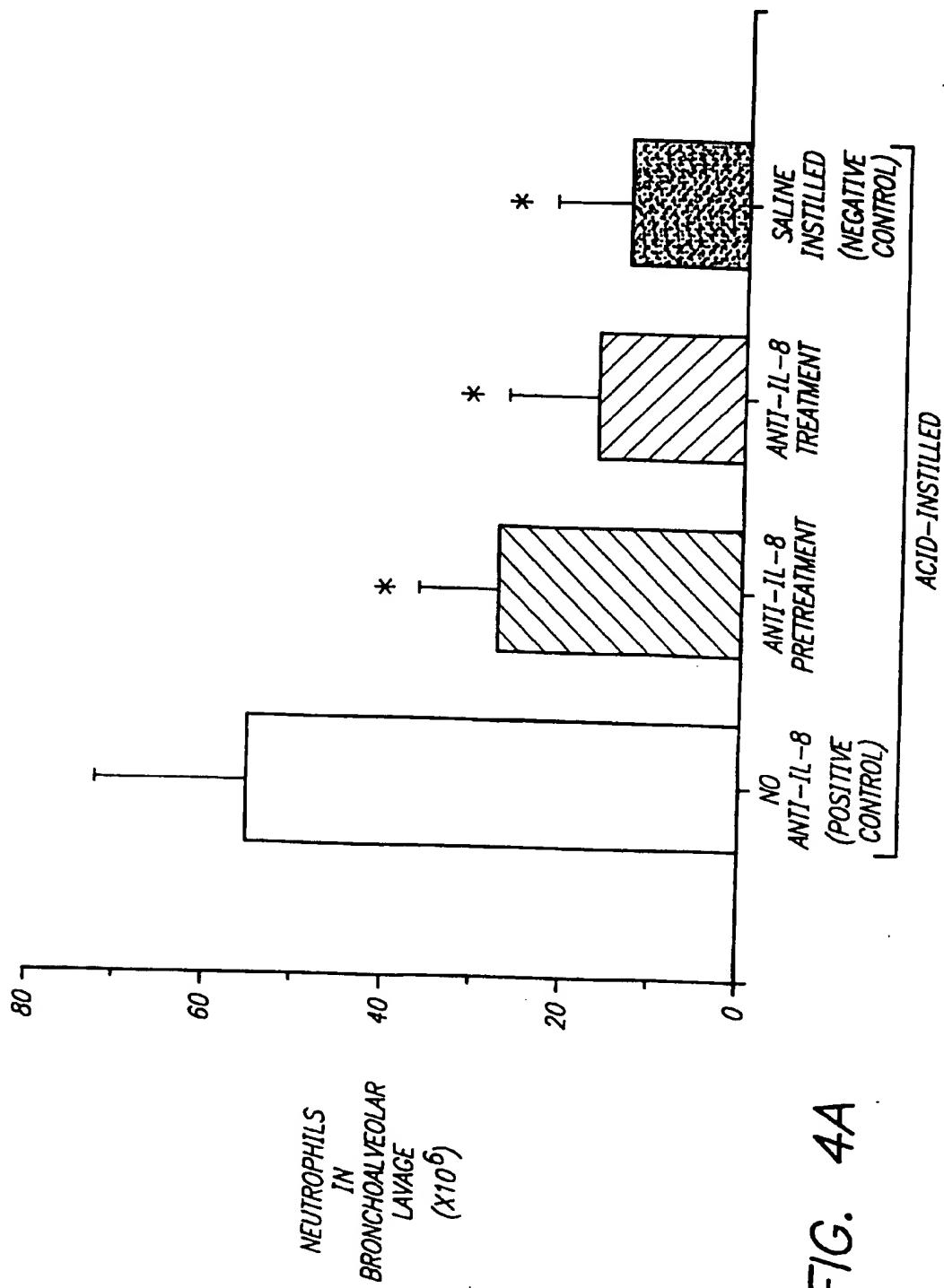
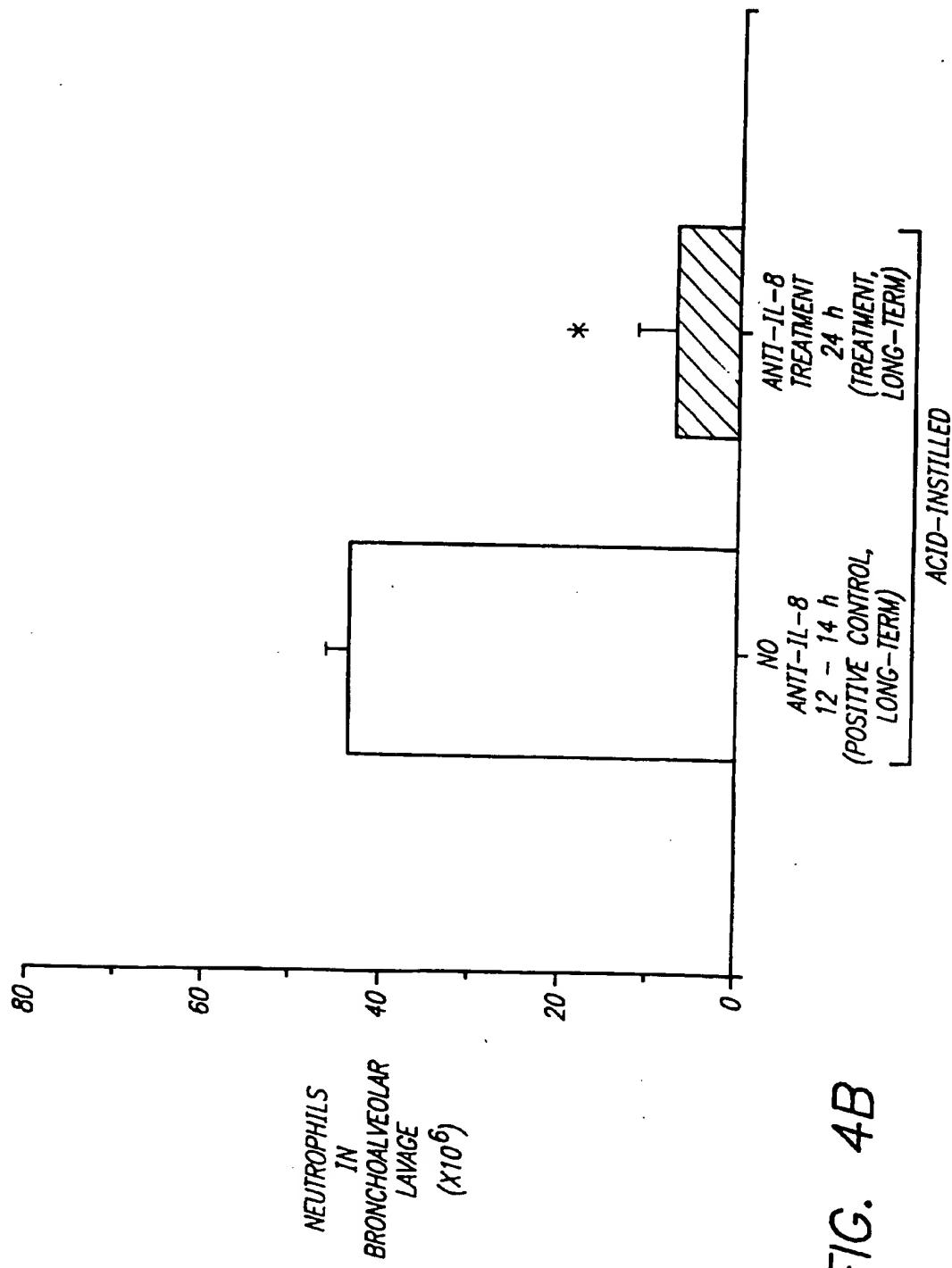


FIG. 4A

8/8



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01150

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/131.1, 145.1, 158.1, 172.1; 514/2; 530/350, 388.23, 389.2, 351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, DERWENT WPI, CHEM AB, APS search terms: author names, IL-8, lungs, acid, antibody, anti IL-8, receptor, acid, neutrophils, binding

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEKIDO et al. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. Nature. 14 October 1993, Vol. 365, pages 654-657, see entire document.	1-6
Y	GOLDMAN et al. Tumor necrosis Factor-alpha mediates acid aspiration-induced systemic organ injury. Ann. Surg. October 1990, vol. 212, pages 513-520, see entire document.	1-6

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
* Special categories of cited documents:		T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A	document defining the general state of the art which is not considered to be of particular relevance	X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E	earlier document published on or after the international filing date	Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, i.e. exhibition or other means	*&*	document member of the same patent family
P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
08 MAY 1996	13 MAY 1996

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Hilary M. Fuerst</i> RON SCHWADRON Telephone No. (703) 308-0196
---	---

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01150

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 38/16, 39/395; C07K 14/00, 14/715, 14/435, 16/00, 16/24, 16/28

A. CLASSIFICATION OF SUBJECT MATTER:
US CL.:

424/131.1, 145.1, 158.1, 172.1; 514/2; 530/350, 388.23, 389.2, 351